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The effects of environmental and individual quality on reproductive performance

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Chapter 2

Influence of fine-scale habitat structure on nest-site occupancy, laying date and clutch size in blue tits *Cyanistes caeruleus*

Seyed Mehdi Amininasab, Oscar Vedder, Elske Schut, Berber de Jong, Michael J. L. Magrath, Peter Korsten & Jan Komdeur

Abstract

Most birds have specific habitat requirements for breeding. The vegetation structure surrounding nest-sites is an important component of habitat quality, and can have large effects on avian breeding performance. We studied 13 years of blue tit *Cyanistes caeruleus* population data to determine whether characteristics of vegetation structure predict site occupancy, laying date and number of eggs laid. Measurements of vegetation structure included the density of English oak *Quercus robur*, European beech *Fagus sylvatica*, and other deciduous, coniferous and non-coniferous evergreen trees, within a 20-m radius of nest-boxes used for breeding. Trees were further sub-divided into specific classes of trunk circumferences to determine the densities for different maturity levels. Based on Principal Component Analysis (PCA), we reduced the total number of 17 measured vegetation variables to 7 main categories, which we used for further analyses. We found that the occupancy rate of sites and the number of eggs laid correlated positively with the proportion of deciduous trees and negatively with the density of coniferous trees. Laying of the first egg was advanced with a greater proportion of deciduous trees. Among deciduous trees, the English oak appeared to be most important, as a higher density of more mature English oak trees was associated with more frequent nest-box occupancy, a larger number of eggs laid, and an earlier laying start. Furthermore, laying started earlier and more eggs were laid in nest-boxes with higher occupancy rates. Together, these findings highlight the role of deciduous trees, particularly more mature English oak, as important predictors of high-quality preferred habitat. These results aid in defining habitat quality and will facilitate future studies on the importance of environmental quality for breeding performance.

1- Introduction

In many birds, especially territorial species, one of the main determinants of fitness is the quality of the breeding habitat (e.g. Davenport et al., 2000; Langen & Vehrencamp, 1998; Pärt, 2001; Sergio et al., 2009). In the last few decades, measuring habitat quality–fitness relationships has posed a challenge of major importance for ecologists because of the need to consider biodiversity values for conservation of birds species, and also for landscape managers because of increasing anthropogenic influences and habitat loss (Sih et al., 2000). Ecologists have used different indices to estimate habitat quality for birds (see e.g. Chandler & King, 2011; Janiszewski et al., 2013), and one of these indices is the vegetation structure around nest-sites (e.g. Atiénzar et al., 2009, 2010; Bell et al., 2014; Wilkin et al., 2007, 2009). Both experimental and observational studies have demonstrated that the vegetation structure around nest-sites has an influence on breeding performance (e.g. Pärt, 2001; Arriero et al., 2006). Especially in insectivorous species of birds, the vegetation is one of the main environmental factors affecting food availability in forest habitats (van Balen, 1973; Blondel et al., 1991). Higher food availability during brood-rearing increases offspring growth and survival (Kroll & Haufler, 2007; Mägi et al., 2009). Thorough knowledge about these relationships can have implications for effective conservation of biodiversity, harvest management in forestry and also reforestation plans (Arriero et al., 2006).

In this study, we used 13 years of breeding data of a population of blue tits *Cyanistes caeruleus*, to evaluate the effect of vegetation structure on nest-site occupancy and two key parameters of breeding performance closely linked to fitness: laying date and clutch size. Blue tits are small, territorial passerines, typically breeding in pairs. While they breed in heterogeneous environments and in a variety of forest types, which differ in quality (e.g. Lambrechts et al., 2004), they are often considered Oak forest specialists (e.g. Perrins, 1979; Lambrechts et al., 2004; Blondel et al., 2006). Parents feed their chicks with invertebrates, particularly caterpillars, which are the main food item available during the brood-rearing phase (Perrins, 1991; Cholewa & Wesolowski, 2011). Availability of caterpillars and other arthropods depends on the timing of spring growth of leaves, which differs between tree species (Blondel et al., 1993). Tits (*Paridae* family) are sensitive to small differences in vegetation structure (Wilkin et al., 2007; Atiénzar et al., 2010) and also suffer reduced breeding performance in human-modified habitats (Hinsley et al., 2009). Hence, they can serve as a good model species to evaluate habitat quality effects on breeding performance (Blondel et al., 1993), and to guide landscape management.

Due to experiments performed in this population during the incubation and nestling stages, we were not able to incorporate other parameters of breeding performance (e.g. number of fledglings). Laying date and clutch size are often correlated with other parameters of breeding performance, such as number of fledged offspring, and subsequent offspring survival and recruitment (e.g. Perrins, 1965; Norris, 1993; Verboven & Visser, 1998; Both et al., 2000). Observational and experimental studies have also indicated that laying date and clutch size are influenced by food availability (Blondel et al., 1991; Nager et al., 1997; Bourgault et al., 2009; Smith et al., 2013). Site occupancy is one of the most important basic predictors that over the long-term reflects habitat quality (Sergio & Newton, 2003; Janiszewski et al., 2013).

To investigate the relationship between the habitat and these parameters of breeding performance (laying date and clutch size) as well as nest-site occupancy, we studied the characteristics of the trees, including their maturity, in the near vicinity of nest-boxes. Although previous studies have investigated the relationship between vegetation and various measures of breeding performance in blue tits and great tits *Parus major*, most of them have studied this on a coarser scale, for example by comparing parameters of breeding performance between deciduous and coniferous forests (e.g. Blondel et al., 1993; Mägi et al., 2009). Some more detailed and finer-scale studies have also investigated the relationship between parameters of breeding performance and the local densities of a single tree species, like food-rich oak trees (Wilkin et al., 2009; Bell et al., 2014). However, comprehensive fine-scale studies that simultaneously investigate several vegetation characteristics, including both the local densities and maturity levels of trees of different species surrounding the nest-sites, and relate these detailed vegetation characteristics to parameters of breeding performance are scarce (for exceptions see Arriero et al., 2006; Hinsley et al., 2009; Atiénzar et al., 2010). Specifically, the objectives of this paper are to examine the relationship between fine-scale vegetation characteristics, particularly the local densities and maturity of different tree species, and 1) nest-site occupancy, 2) laying date and 3) the number of eggs laid. Based on these analyses, we aim to identify the main vegetation indices as predictors of habitat quality.

2- Material and methods

2-1- Study area and study population

The study was conducted over 13 consecutive breeding seasons (2001-2013) on a nest-box breeding blue tit population at 'De Vosbergen' estate near Groningen in the North of The Netherlands (53°N, 06°E). The 54 ha study area consists of

mixed deciduous and coniferous forest interspersed with areas of open grassland. The study area contains about 188 wooden nest-boxes (inner dimensions: ca. $9.0 \times 12.5 \times 25$ cm, with a 2.6 cm entrance hole) designed especially for blue tits. Nest-boxes have been installed in excess in the study area since 2001 and are dispersed at relatively regular intervals over the whole study area.

2-2- Breeding performance

Every breeding season (April–June), for each nest-box, breeding activities from the nest-building until the fledging phase were monitored. During the nest-building phase from the beginning of April all nest-boxes were checked regularly. For each blue tit breeding pair, we recorded the date of laying the first egg (hereafter referred to as ‘laying date’) and the number of eggs laid.

Various studies and experiments were carried out on this population during the years of monitoring, which may have influenced different parameters of breeding performance (see Korsten et al., 2006; Kingma et al., 2009; Vedder et al., 2010, 2012; de Jong, 2013; Schut et al., 2014). Some experiments were carried out during or before the onset of laying and some of them were carried out after the onset of incubation. Because of this, we did not use breeding performance parameters determined after clutch completion, and only selected data which were not influenced by experiments (see Supplementary material, Table S1). The selected parameters were: nest-box occupancy, laying date, and number of eggs laid. A nest-box was considered occupied in a particular year when it contained a nest with at least one egg. The occupancy rate was calculated for each nest-box on the basis of its availability (on rare occasions nest-boxes were occupied by other species such as great tits, and coal tits *Parus ater*, or nest-boxes were absent or damaged and could not be used for breeding that year) and number of years occupied during the 13-year period. Laying date was defined as the date of laying the first egg (1 April = day 1). Number of eggs was the total of all eggs laid. As a result of collection of eggs in some years without replacement with dummy eggs (see Supplementary material, Table S1), it was not possible to determine the originally intended clutch size, as females may, or may not, have compensated by laying additional eggs. For these years, the eggs were collected for most of the clutches in the population leaving the within-year comparisons meaningful. In recognition of this potential influence we use the term number of eggs laid instead of clutch size. We excluded second clutches, which occurred only rarely, as well as replacement clutches after failed first breeding attempts, from our analyses.

Experiments had an influence on the observed laying date and the number of eggs laid in 2006-2007 (unpublished results) and also on the number of eggs laid

in 2009 (Vedder et al., 2012; for details see Supplementary material, Table S1). As a result, for those years and breeding performance parameters, we only included control-treated nest-boxes in our analyses.

2-3- Measurements of habitat characteristics

The vegetation structure of the habitat was measured by sampling the trees surrounding the nest-boxes. Tree sampling took place during two periods; the vegetation around 100 of the nest-boxes was sampled between 2002 and 2004, and around the remaining 88 nest-boxes in 2013. Because of the large time gap between the two sampling periods, we repeated the tree sampling for 20 randomly chosen nest-boxes in 2013 that had also been sampled between 2002 and 2004 and compared the vegetation values between the two sampling periods. All vegetation variables analysed were significantly correlated between sampling periods ($0.51 \leq r \leq 0.92$, $P \leq 0.02$; Pearson's correlations), which indicates relatively minor changes in vegetation structure between the two sampling periods. As a result, vegetation measurements of both periods were used in the analyses. We used the measurements taken during 2002-2004 for 80 nest-boxes, and measurements taken in 2013 for 108 nest-boxes.

In evaluating the radius around each nest-box in which vegetation structure may be relevant, we found different distance estimates in the literature on blue tits and great tits. For example, Stauss et al. (2005) reported average flight distances of blue tits between nest-boxes and foraging patches of about 22 m in preferred deciduous habitat. In other literature, the distance to foraging patches for great tits is given as limited to 45 m in 90% of the foraging trips (Naef-Daenzer & Keller, 1999). Another study in blue tits found average foraging distances during brood rearing of 25 m and 53 m in two different habitats with rich and poor food abundance, respectively. Remarkably, in one of the two areas, which had evergreen vegetation and hence poor caterpillar abundance, individual blue tits regularly flew more than 50 m, with even a few recordings of distances of >500 m (Tremblay et al., 2005). In some studies, researchers measured the habitat characteristics within 50 m from the nest-boxes for both great tits (Wilkin et al., 2009) and blue tits (Bell et al., 2014). The foraging distance may also vary during the different stages of the breeding cycle. For example, females may forage at much longer distances from the nest-box during the period of egg formation than during the incubation and the brood rearing stages, because during the latter stages they have to return frequently to the nest to incubate the eggs or feed the nestlings. In spite of this variation in distances, in this study a radius of 20 m was chosen, as a compromise between biological plausibility and practical feasibility given the

workload of counting and measuring trees. Furthermore, we assume that the vegetation structure estimates taken at a small spatial scale (a radius of 20 m) are representative for the vegetation at a somewhat larger scale surrounding the nest-boxes.

Within the 20 m radius, we sampled and identified the species of all living trees with a minimal circumference of 30 cm. Trees with thinner trunks were excluded as they were not considered to harbour a substantial amount of food for breeding blue tits. The trees' circumference (cm) was measured about 130 cm from the ground, using a measuring tape.

2-3-1- Classification of trees

We identified and measured 8442 trees surrounding 188 nest-boxes (including 80 nest-boxes sampled between 2002 and 2004 and 108 nest-boxes sampled in 2013; Table 1). The most dominant species surrounding the nest-boxes were English oak *Quercus robur* ($n = 2168$) and European beech *Fagus sylvatica* ($n = 2092$). Trees were divided into five main categories: 1) English oak, a deciduous species, the most dominant tree species in the area, and an important source of the main prey item, caterpillars, during the breeding season; 2) European beech, a deciduous species, and the second dominant species in the area; 3) Miscellaneous deciduous ($n = 2129$): deciduous species other than English Oak and European Beech; for example European white birch *Betula pubescens* ($n = 350$), Northern red oak *Quercus rubra* ($n = 230$), and sycamore maple *Acer pseudoplatanus* ($n = 202$) were included in this category. Deciduous trees are known as preferred breeding habitat for great tits and blue tits; 4) Coniferous trees ($n = 1720$); for example Douglas fir *Pseudotsuga menziesii* ($n = 444$), Scots pine *Pinus sylvestris* ($n = 380$), and Norway spruce *Picea abies* ($n = 300$); and 5) non-coniferous evergreen ($n = 333$); for example holly *Ilex aquifolium*. Each of the five tree categories were further subdivided based upon trunk circumferences (Table 1), as an index of the maturity of the trees.

2-4- Statistical analyses

In the analyses, the vegetation variables were used as independent variables, and occupancy rate and breeding performance parameters (laying date, clutch size) were used as response variables. Similar to the approach by Wilkin et al. (2007), Atienzar et al. (2010), Maícas et al. (2012) and Bell et al. (2014), we used the counts (density) of trees in the different categories surrounding nest-boxes (Table 1) as vegetation variables. (In addition to the density of trees in the different categories, we separately used the proportion of deciduous trees, as broad-scale

index of habitat quality, defined as the number of all deciduous trees divided by the total number of all trees, in the 20-m radius around the nest-box).

Statistical models were implemented in R (version 3.1.0; R Development Core Team, 2014). Based on principal component analysis (PCA) we reduced the total number of vegetation variables (17 vegetation variables, see Table 1) to a smaller number of variables by merging different categories, while minimizing the loss of independent information. We used this reduced set of 7 variables as final vegetation variables in our further analyses.

Two types of models were used to analyse the relationships between vegetation variables and nest-box occupancy and breeding performance parameters. First, we applied generalized linear mixed models (GLMMs) with binomial error distribution and a logit link function using the lme4 package (version 1.1-7; Bates et al., 2014) to test the relationships between nest-box occupancy (response variable) and the vegetation variables (independent variables). Second, to determine whether vegetation variables and occupancy rate were related to laying date and number of eggs laid, we used linear mixed models (LMMs) with normally distributed errors and an identity link function, also using the package lme4. Given that the residuals of the models with the number of eggs laid (response variable) followed a normal distribution, and generalized linear mixed models with Poisson errors and a log link function failed to converge, we used models with normally distributed errors for fitting the number of eggs laid, instead of using Poisson errors, which are often used for count data. In both sets of models, we also included year and nest-box identity as random effects to statistically control for between-year variation and potential non-independence of multiple observations at the same nest-box. Maximal models (i.e. including all vegetation variables) with estimated coefficients (with associated standard errors, and t or Z and P values) are presented. P values < 0.05 were considered significant.

Table 1 Frequency of trees and actual mean (\pm SD) circumference for the different tree categories.

Tree category	Tree circumference class (cm)	Number of trees	Actual circumference (cm, mean \pm SD)
English oak		2168	122.7 \pm 54.3
	30-50	217	39.4 \pm 6.0
	50-100	477	74.0 \pm 15.4
	100-200	1297	139.1 \pm 25.0
	>200	177	235.9 \pm 32.7
European beech		2092	94.2 \pm 62.6
	30-50	627	38.6 \pm 5.8
	50-100	711	71.2 \pm 14.1
	100-200	607	141.3 \pm 27.3
	>200	147	247.8 \pm 49.5
Miscellaneous deciduous		2129	63.1 \pm 43.0
	30-50	1160	38.1 \pm 5.7
	50-100	687	66.7 \pm 13.4
	100-200	235	142.3 \pm 29.2
	>200	47	231.5 \pm 26.2
Coniferous		1720	106.5 \pm 46.1
	30-50	227	38.8 \pm 5.7
	50-100	548	77.7 \pm 14.6
	100-200	892	134.1 \pm 24.0
	>200	53	228.7 \pm 31.4
Non-coniferous evergreen		333	44.7 \pm 14.7

3- Results

3-1- Laying date and number of eggs laid

There were significant differences between years, both for the laying date (ANOVA, $n = 1216$, $F_{12,1203} = 97.27$, $P < 0.001$), and for the number of eggs laid (ANOVA, $n = 1144$, $F_{12,1131} = 7.06$, $P < 0.001$). During the 13 years of study, mean laying date was earliest in 2012 (around 12th April) and latest in 2013 (around 30th April). The (annual mean) minimum and maximum number of eggs

per clutch were laid in 2011 (10.4 eggs) and 2008 (12.6 eggs), respectively (Supplementary material, Fig. S1).

3-2- Reducing the number of vegetation variables based on principal component analysis

Principal components analysis (PCA) was carried out on seventeen directly measured vegetation variables (Table 1) to find the main components summarizing the vegetation composition. The first ten principal components explained 83.3% of total variance in the measured vegetation variables, with a relatively small percentage of variance explained by the remaining components (Supplementary material, Table S2). According to these results and the relative importance of the different components (Supplementary material, Fig. S2), PC1 and PC2 were considered as the most important axes of vegetation variation, explaining 15.9% and 14.4% of the variance, respectively (30.3% of the total variance across all measured vegetation variables). Plotting the measured vegetation variables for each nest-box along these two axes (Fig. 1), allowed us to reduce the number of vegetation variables from 17 to 7, by merging different vegetation variables according to visual interpretation of Fig. 1. This left us with the following variables: (1) Young English oak trees (30-100 cm); (2) Mature English oak trees (> 100 cm); (3) European beech trees (> 30 cm); (4) Young miscellaneous deciduous trees (30-100 cm); (5) Mature miscellaneous deciduous trees (> 100 cm); (6) Coniferous trees (> 30 cm); and (7) Non-coniferous evergreen trees (> 30 cm). These seven variables were used as final vegetation variables in the models (see Table S3 for the correlations between the 7 vegetation variables and the first and second principle components resulting from the PCA analysis and Table S4 for the correlation matrix for these newly constructed variables).

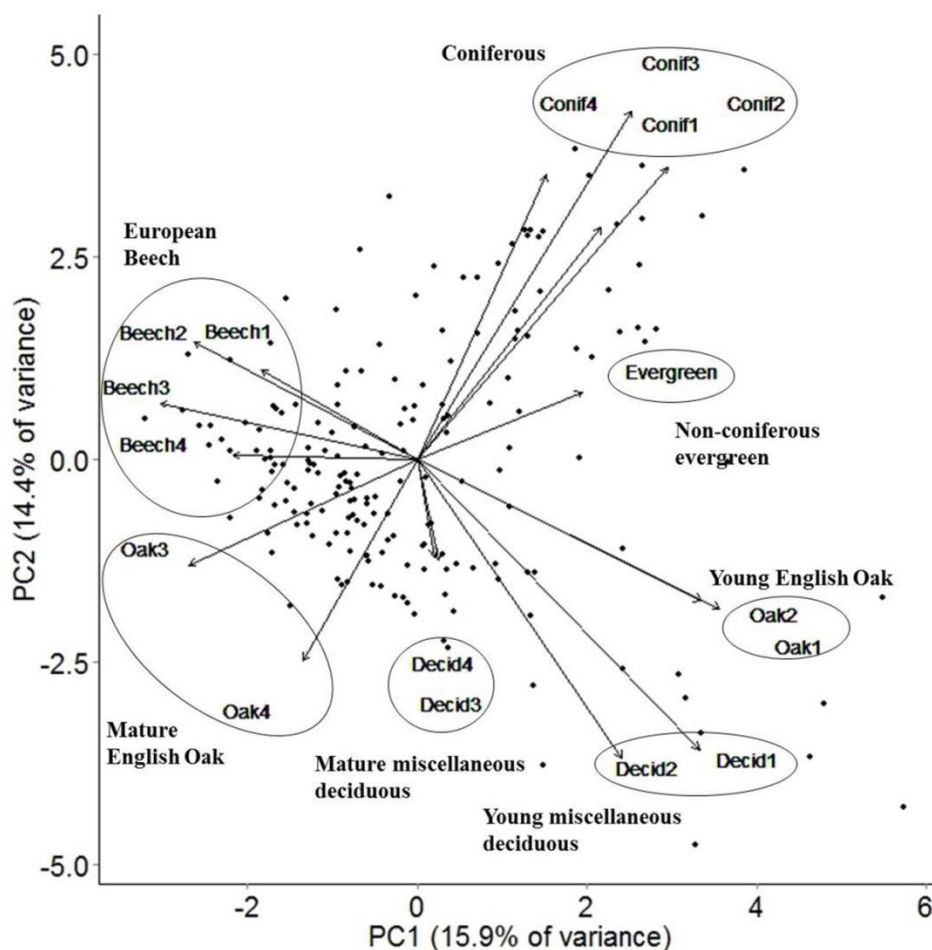


Figure 1 Biplot of the seventeen variables of tree density along the first and second principal component vectors. The number of vegetation variables was reduced from 17 to 7 final variables (shown in circles) on the basis of the multidimensional distribution of tree densities for the different categories. Code number represents the range of tree circumferences for each of the measured categories; 1: 30-50 cm, 2: 50-100 cm, 3: 100-200 cm, 4: > 200 cm.

3-3- Nest-box occupancy in relation to vegetation characteristics

Blue tits occupied nest-boxes significantly more frequently if deciduous trees made up a greater proportion of all trees surrounding the nest-box (Estimate \pm SE = 1.958 ± 0.259 , $Z = 7.565$, $P < 0.001$, $n = 2405$, random effects of nest-box and year: variance = 0.45, 0.35, respectively; Fig. 2a). Blue tits occupied nest-boxes less frequently with greater densities of surrounding coniferous and European

beech trees (Table 2). The density of young miscellaneous deciduous trees had a positive effect on the occupancy rate (Table 2). We found no significant relationships between nest-box occupancy and density for the other tree categories. However, there was a negative correlation between the density of coniferous and the density of mature English oak trees in the habitat surrounding nest-boxes (Supplementary material Table S4). Hence, in habitats with more coniferous trees, there were fewer mature oak trees. We therefore tested an alternative simpler model, which only included the densities of the two classes of English oaks (young and mature) as predictors. This yielded a positive association between nest-box occupancy rate and the density of mature English oaks (Estimate \pm SE = 0.042 ± 0.012 , $Z = 3.578$, $P < 0.001$, $n = 2405$, random effects of nest-box and year: variance = 0.64, 0.35, respectively), while there was no significant association between occupancy rate and the density of young English oaks (Estimate \pm SE = -0.007 ± 0.011 , $Z = -0.653$, $P = 0.51$).

3-4- Laying date and number of eggs laid in relation to vegetation characteristics

The laying date was advanced with a greater proportion of deciduous trees among all surrounding trees (Estimate \pm SE = -3.470 ± 0.736 , $t = -4.715$, $P < 0.001$, $n = 1216$, random effects of nest-box and year: variance = 2.06, 27.87, respectively; Fig. 2b). Laying started earlier when the densities of mature English oak trees and young miscellaneous deciduous trees were higher (Table 3). Furthermore, laying started later when the density of coniferous trees was higher (Table 3).

More eggs were laid when the proportion of deciduous trees surrounding the occupied nest-boxes was higher (Estimate \pm SE = 1.671 ± 0.272 , $t = 6.134$, $P < 0.001$, $n = 1144$, random effects of nest-box and year: variance = 0.19, 0.26, respectively; Fig. 2c). The number of eggs laid was positively correlated with the density of mature English oak trees surrounding the nest-boxes (Table 4), and negatively correlated with the density of coniferous trees (Table 4).

Table 2 Results of generalized linear mixed model (with binomial error distribution) for the relationships between nest-box occupancy and vegetation variables. Estimated coefficients (with associated standard errors, *Z* and *P* values) of significant variables are in bold.

Vegetation variables	Estimate	Nest-box occupancy		
		SE	Z value	P value
Fixed effects				
(Intercept)	0.658	0.270	2.434	0.014
Young English oak	-0.019	0.011	-1.741	0.081
Mature English oak	0.015	0.012	1.256	0.21
European beech	-0.013	0.007	-1.984	0.047
Young miscellaneous deciduous	0.012	0.006	2.010	0.044
Mature miscellaneous deciduous	0.004	0.020	0.182	0.85
Coniferous	-0.034	0.006	-5.419	< 0.001
Non-coniferous evergreen	-0.035	0.018	-1.904	0.056
Random effects		Variance		
Nest-box		0.41		
Year		0.35		
Sample sizes				
Years		13		
Nest-boxes		188		
Number of observations		2405		

Table 3 Results of linear mixed model for the relationships between laying date and vegetation variables. Estimated coefficients (with associated standard errors, *t* and *P* values) of significant variables are in bold.

Vegetation variables	Estimate	SE	Laying date	<i>P</i> value
			<i>t</i> value	
Fixed effects				
(Intercept)	19.654	1.562	12.580	< 0.001
Young English oak	-0.003	0.029	-0.111	0.91
Mature English oak	-0.120	0.030	-4.147	< 0.001
European beech	0.009	0.018	0.508	0.61
Young miscellaneous deciduous	-0.036	0.014	-2.626	0.009
Mature miscellaneous deciduous	0.061	0.051	1.202	0.23
Coniferous	0.036	0.017	2.073	0.039
Non-coniferous evergreen	0.032	0.050	0.634	0.53
Random effects		Variance		
Nest-box		1.53		
Year		27.82		
Residual		23.18		
Sample sizes				
Years		13		
Nest-boxes		188		
Number of observations		1216		

Table 4 Results of linear mixed model for the relationships between number of eggs laid and vegetation variables. Estimated coefficients (with associated standard errors, *t* and *P* values) of significant variables are in bold.

Vegetation variables	Estimate	Number of eggs laid		
		SE	<i>t</i> value	<i>P</i> value
Fixed effects				
(Intercept)	11.189	0.251	44.428	< 0.001
Young English oak	0.011	0.011	1.002	0.32
Mature English oak	0.028	0.011	2.455	0.015
European beech	-0.008	0.007	-1.130	0.26
Young miscellaneous deciduous	0.006	0.005	1.075	0.28
Mature miscellaneous deciduous	-0.003	0.019	-0.161	0.87
Coniferous	-0.029	0.007	-4.303	< 0.001
Non-coniferous evergreen	0.005	0.020	0.269	0.79
Random effects		Variance		
Nest-box		0.19		
Year		0.25		
Residual		3.47		
Sample sizes				
Years		13		
Nest-boxes		188		
Number of observations		1144		

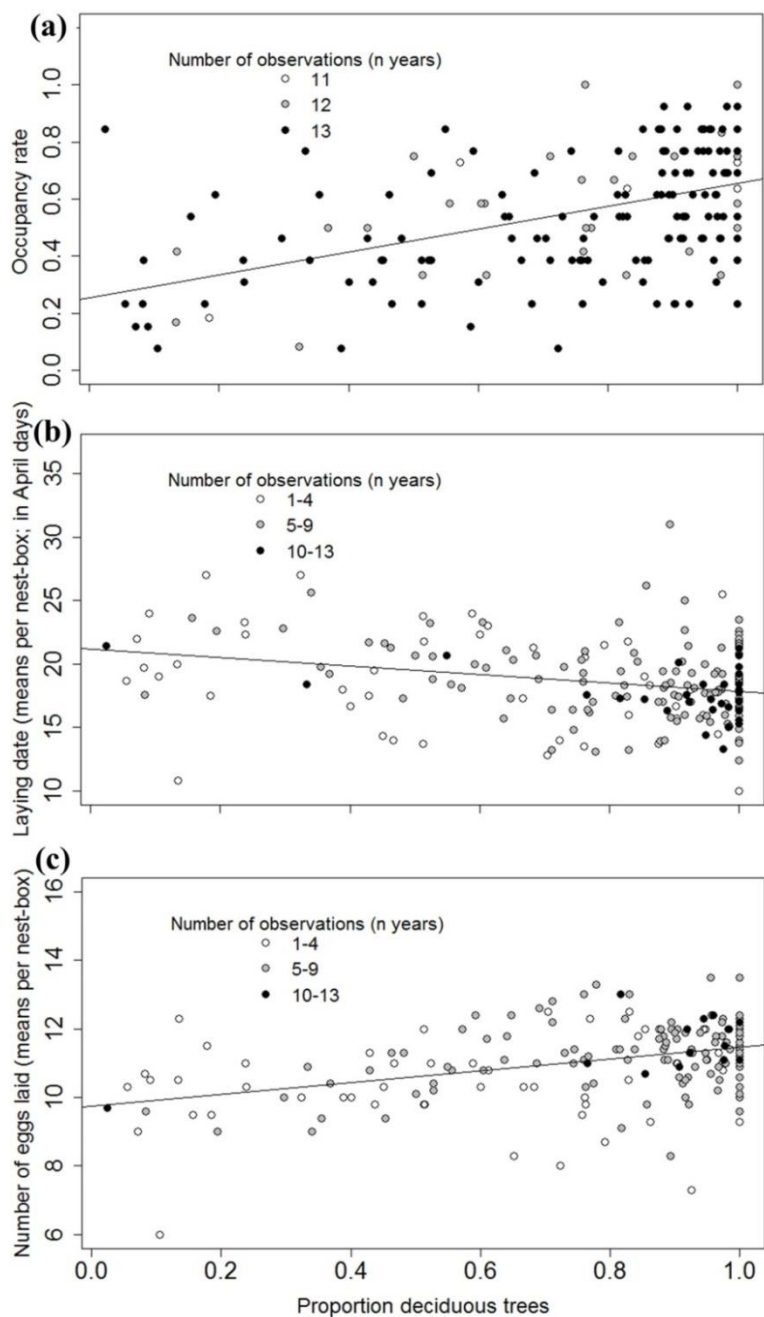


Figure 2 The relationships between the proportion of deciduous trees and the (a) occupancy rates of nest-boxes (b) mean laying date; and (c) mean number of eggs laid during the period (2001-2013). The trend lines are least-squares regression lines added for visual purpose only, and do not represent the model estimates that are presented in the text.

3-5- Laying date and number of eggs laid in relation to occupancy rate of a nest-box

We found that laying date was negatively associated with occupancy rate (Estimate \pm SE = -3.665 ± 0.906 , $t = -4.046$, $P < 0.001$, $n = 1216$, random effects of nest-box and year: variance = 2.20, 28.04, respectively; Fig. 3a). Furthermore, the number of eggs laid was positively associated with occupancy rate (Estimate \pm SE = 1.754 ± 0.343 , $t = 5.109$, $P < 0.001$, $n = 1144$, random effects of nest-box and year: variance = 0.26, 0.25, respectively; Fig. 3b). Hence, in nest-boxes that were more frequently occupied across years, laying started earlier and more eggs were laid.

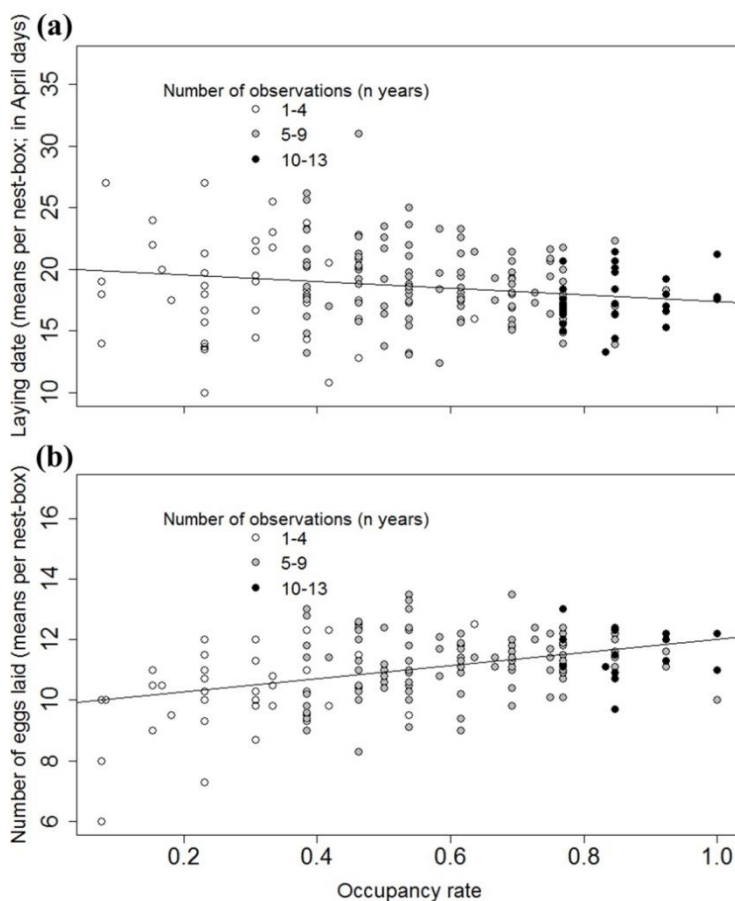


Figure 3 The relationships between the occupancy rates of nest-boxes and their mean values for (a) laying date; (b) number of eggs laid during the study period (2001-2013). The trend lines are least-squares regression lines added for visual purpose only, and do not represent the model predictions that are presented in the text.

4- Discussion

4-1- Nest-box occupancy in relation to vegetation characteristics

By linking nest-box occupancy to vegetation characteristics, we showed that nest-box occupancy can act as a good predictor for the quality of the habitat. The probability of nest-box occupancy was higher when the proportion of deciduous trees and young miscellaneous deciduous trees surrounding the nest-box increased. Conversely, negative correlations between the density of coniferous and European beech trees surrounding the nest-boxes and occupancy rate suggest that these tree species are indicators of poor-quality, non-preferred habitats. However, because there are negative correlations between the densities of different tree categories, we cannot exclude the importance of mature English oaks, which other studies have identified for great tits (Mänd et al., 2005; Wilkin et al., 2009). Indeed, an alternative model that only included young and mature English oaks as predictors, suggests that the density of mature English oaks can also act as a good predictor of preferred high quality habitat in blue tits. Yet, we cannot exclude the possibility that in blue tit and great tit populations are adapted to quite different vegetation types (e.g. see Tremblay et al., 2003; Mägi et al., 2009) and that different tree species may be better predictors of habitat quality for each of the two species. Overall our results are consistent with the findings of Sergio & Newton (2003) and Janiszewski et al. (2013), who suggest that nest-box occupancy rate in the long-term can be a simple and reliable indicator of habitat quality with relatively high potential for application to territorial species.

4-2- Laying date and number of eggs laid in relation to vegetation characteristics

We found that laying date and number of eggs laid were associated with the local vegetation characteristics, and thus the distribution of a particular tree species. Laying started earlier and a larger number of eggs were laid in deciduous habitats and in particular in areas with a higher density of English oak trees. The finding of habitat-related laying date is consistent with other studies on blue tits (Blondel et al., 1993; Lambrechts et al., 2004; Arriero et al., 2006; Thorley & Lord, 2015) and great tits (Mänd et al., 2005; Wilkin et al., 2007; Mägi et al., 2009; Atienzar et al., 2010). Similarly, our finding that clutch size was related to vegetation variables has also been reported in other studies on blue tits (Blondel et al., 1993; Tremblay et al., 2003; Bell et al., 2014; but see Arriero et al. (2006) where no association was found) and great tits (Belda et al., 1998; Mänd et al., 2005; Mägi et al., 2009).

The proximate link of laying date and number of eggs laid with vegetation structure may be differences in food availability in trees of different species and

maturity surrounding breeding sites. For example, Svensson & Nilsson (1995) have shown in blue tits that experimental food supplementation prior to laying causes an advance in laying date. Although we have no data about food abundance in the trees surrounding the nest-boxes in our study area, other studies suggest that food availability varies between different vegetation types (van Balen, 1973; Perrins, 1991; Lambrechts et al., 1997). For example, deciduous trees, which produce new soft leaves each spring, support more caterpillars than coniferous trees (Blondel et al., 1993; Lambrechts et al., 2004; Mänd et al., 2005). Among deciduous trees, oaks are the most important food source, as they harbour more caterpillars and higher densities of other invertebrates than other deciduous tree species, which may enable birds in oak habitats to start breeding earlier (Blondel et al., 1991; Wilkin et al., 2007). Other studies have shown that leaves of coniferous trees are not edible by insects due to their hardness and biochemical compounds such as tannins (Feeny, 1970; Hatcher, 1990). The peak of insect abundance may also occur later in coniferous trees (Rytönen & Orell, 2001). Food availability can be an important constraint on energy expenditure for females, particularly early in the season (Tinbergen & Williams, 2002). Vegetation type-related variation in food availability may therefore have a greater influence on breeding parameters expressed at earlier stages of the reproductive cycle, such as laying date (Gienapp & Visser, 2006; Bourgault et al., 2009) and clutch size (Perrins, 1965; Marciniak et al., 2007).

In addition to the density of specific tree species surrounding the nest-boxes, their size and maturity also influence food availability within habitats (Arriero et al., 2006). We found that laying started earlier in nest-boxes surrounded by young miscellaneous deciduous trees. These findings did not agree with our expectation, because young miscellaneous deciduous trees should provide less foliage and associated food for blue tits. The density of young miscellaneous deciduous trees was positively correlated with the density of young English oaks (Supplementary material Table S4) surrounding the nest-boxes. Presumably, the high abundance of these young trees may provide enough food resources for blue tits. We also found that in habitats with more mature English oaks, egg laying started earlier and the number of eggs was greater. This suggests that, mature English oaks may provide greater food resources (e.g. caterpillars).

To assess the exact fitness consequences of the habitat structure around the nest-sites, complete measures of reproductive success would be required. Due to the experiments performed in this population (see Korsten et al., 2006; Kingma et al., 2009; Vedder et al., 2010, 2012; de Jong, 2013; Schut et al., 2014) we were not able to look at vegetation effects on breeding performance beyond the number of

eggs laid. However, in blue tits and great tits, clutch size is generally found to be positively correlated with other parameters of breeding performance such as hatching success (e.g. Arriero et al., 2006), number of fledglings (e.g. Lambrechts et al., 2004; Ahola et al., 2009) and local recruitment (e.g. Boyce & Perrins, 1987). But to ascertain that this is also the case in our study population, more research is needed on the relationship between habitat characteristics and other measures of breeding performance, like hatching and fledging success, and subsequent offspring survival and recruitment.

4-3- Laying date and number of eggs laid in relation to nest-box occupancy

The relationships among nest-box occupancy, vegetation characteristics, and breeding parameters (laying date and number of eggs laid) were clearly congruent. We found earlier laying and a larger number of eggs laid in the nest-boxes that were more frequently occupied across the 13 years of research. Similarly, in great tits it was found that if a nest-box was more frequently occupied across years, clutches in that nest-box were laid earlier in the year and were larger (Mänd et al., 2005).

Occupancy rate has previously been suggested to indicate the quality and quantity of food resources surrounding nest-sites (Sergio & Newton, 2003; Janiszewski et al., 2013). Accordingly, we suggest that in our population nest-sites in higher-quality habitat with a higher proportion of deciduous vegetation and a relatively high density of mature English Oaks – which likely allows earlier breeding and larger clutches – are occupied before the nest-sites in lower-quality habitats (e.g. with coniferous vegetation). While this is also in line with predictions of theoretical studies (Bernstein et al., 1991), more empirical studies on the relationship between occupancy rate and measures of breeding success are required to investigate whether occupancy rate can serve as a simple and general predictor of habitat quality and breeding performance in blue tits and other bird species.

5- Conclusions

We suggest that nest-box occupancy in the long-term can be a simple and reliable predictor of habitat preference and quality. As an immediate index, the proportion of deciduous trees surrounding the nest-site, and the density of English oak trees, particularly mature trees, appear to be good predictors of high quality habitats. Conversely, coniferous trees are indicators of poor-quality, non-preferred habitat. Our results indicate that blue tits preferentially occupy deciduous habitats with a high density of mature English oak trees (and conversely a low density of

coniferous trees), in which they start laying earlier and lay larger clutches. However, we cannot rule out that other blue tit populations may have adapted differently to habitat structure in areas with completely different vegetation.

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Supplementary material

Table S1 Summary of experiments carried out in the study area between 2001-2013 with their possible influences on the laying date and the number of eggs laid.

Year	Experimental treatment ^a	Timing of the experimental treatment with regard to the breeding cycle	Effect on laying date?	Effect on number of eggs laid?	Reference ^b
2001	No experiments	-	-	-	
2002	UV manipulation of male crown plumage	Before egg laying	No	No	Korsten et al., 2006
2003	UV manipulation of male crown plumage	Before egg laying	No	No	Korsten et al., 2006
2004	Removal of nest material	During nest building	No	No	Unpublished results, ($P = 0.15, 0.30$)
2005	Egg collection; replacement with dummy eggs	During egg laying	No	No	Kingma et al., 2009
2006	Addition of nest-boxes near the focal nest-boxes; removal of nesting material; blocking of original nest-box; egg collection and replacement with dummy eggs	During nest building and egg laying	Yes	Yes	Unpublished results, ($P = 0.029, 0.046$)
2007	Addition of nest-boxes near the focal nest-boxes; removal of nesting material; blocking of original nest-box; egg collection and replacement with dummy eggs	During nest building and egg laying	Yes	Yes	Unpublished results, ($P < 0.001, 0.003$)
2008	Egg collection and egg addition	During egg laying	No	No	Vedder et al., 2010
2009	Egg collection; replacement with dummy eggs.	During egg laying	No	Yes	Vedder et al., 2012
2010	Testosterone implants in females and egg collection; replacement with dummy eggs	Before egg laying	No	No	de Jong, 2013
2011	Testosterone implants in females	Before egg laying	No	No	de Jong, 2013
2012	Egg collection	During egg laying	No	Possibly ^c	Personal communication
2013	Egg collection	During egg laying	No	Possibly ^c	Personal communication

^a Only experimental treatments carried out before the incubation onset are presented.

^b The first and second P values in the brackets are related to the effect of the experimental treatment on laying date and number of eggs laid, respectively.

^c In 2012-2013, two eggs per nest were collected without replacement with dummy eggs. This was done for almost all observed breeding attempts. As breeding birds received the same treatment at almost all nest-boxes, we assumed the collection of eggs did not contribute to between-nest-box variation in the number of eggs laid within these years.

Table S2 Loading factors, eigenvalues and variances explained for the first ten principal components resulting from a principal component analysis of vegetation variables.

Vegetation variables	Tree circumference class (cm)	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
English oak	30-50	0.36	-0.18	0.19	-0.20	0.27	-0.26	0.16	-0.08	0.10	-0.05
	50-100	0.33	-0.17	0.29	-0.20	0.18	-0.31	0.13	0.09	-0.02	-0.02
	100-200	-0.27	-0.13	0.36	0.25	-0.15	-0.05	0.09	0.31	-0.50	0.15
	>200	-0.13	-0.25	0.02	0.25	-0.46	0.04	0.17	-0.18	0.55	-0.32
European beech	30-50	-0.18	0.11	0.40	0.06	0.42	0.29	0.01	-0.20	0.18	-0.05
	50-100	-0.26	0.14	0.37	0.07	0.38	0.17	-0.05	0.04	0.26	-0.05
	100-200	-0.30	0.07	-0.14	-0.53	0.01	0.07	0.01	0.06	0.00	-0.07
	>200	-0.21	-0.00	-0.19	-0.58	0.00	0.18	0.13	0.03	0.05	0.02
Miscellaneous deciduous	30-50	0.33	-0.36	0.10	-0.10	-0.07	0.37	-0.13	-0.05	0.02	0.04
	50-100	0.24	-0.37	0.07	-0.08	-0.05	0.45	-0.23	-0.07	-0.09	0.07
	100-200	0.02	-0.12	-0.41	0.21	0.42	-0.01	-0.22	-0.00	-0.24	-0.63
	>200	0.02	-0.12	-0.45	0.27	0.35	0.01	0.09	0.01	0.26	0.63
Coniferous	30-50	0.22	0.28	-0.50	0.09	-0.03	0.50	-0.00	0.44	-0.00	-0.07
	50-100	0.29	0.36	0.06	-0.02	-0.09	-0.10	-0.28	0.38	0.27	0.01
	100-200	0.25	0.43	0.01	-0.02	-0.12	-0.11	-0.09	-0.22	0.06	-0.06
	>200	0.15	0.35	0.01	0.04	-0.06	0.19	0.11	-0.61	-0.33	0.11
Non-coniferous evergreen		0.19	0.08	-0.08	0.06	0.06	0.18	0.82	0.17	-0.05	-0.17
Standard deviation		1.65	1.56	1.30	1.22	1.14	1.07	1.00	0.96	0.88	0.80
	Eigenvalue	2.72	2.43	1.69	1.49	1.30	1.14	1.00	0.92	0.77	0.64
	% variance explained	15.9	14.4	10.0	8.7	7.6	7.00	6.00	5.4	4.5	3.8
	Σ% variance explained	15.9	30.03	40.3	49.0	56.6	63.6	69.6	75	79.5	83.3

Table S3 Pearson’s correlations between the first (PC1) and second (PC2) principal components with densities of trees of the reduced vegetation variables ($n = 188$).

	Young English oak	Mature English oak	European beech	Young miscellaneous deciduous	Mature miscellaneous deciduous	Coniferous	Non-coniferous evergreen
PC1	0.62 ***	-0.47 ***	-0.62 ***	0.53 ***	0.04	0.54 ***	0.32 ***
PC2	-0.31 ***	-0.28 ***	0.23 ***	-0.62 ***	-0.22 **	0.76 ***	0.13

** $P \leq 0.01$, *** ≤ 0.001

Table S4 Pearson’s correlations between the densities of trees for the reduced number of vegetation variables ($n = 188$).

	Young English oak	Mature English oak	European beech	Young miscellaneous deciduous	Mature miscellaneous deciduous	Coniferous	Non-coniferous evergreen
Young English oak	1						
Mature English oak	-0.16 *	1					
European beech	-0.21 **	0.09	1				
Young miscellaneous deciduous	0.35 ***	-0.06	-0.27 ***	1			
Mature miscellaneous deciduous	-0.07	-0.21 **	-0.16 *	0.03	1		
Coniferous	0.03	-0.39 ***	-0.16 *	-0.11	-0.16 *	1	
Non-coniferous evergreen	0.13	-0.11	-0.13	0.02	0.01	0.17 *	1

* $P \leq 0.05$, ** ≤ 0.01 , *** ≤ 0.001

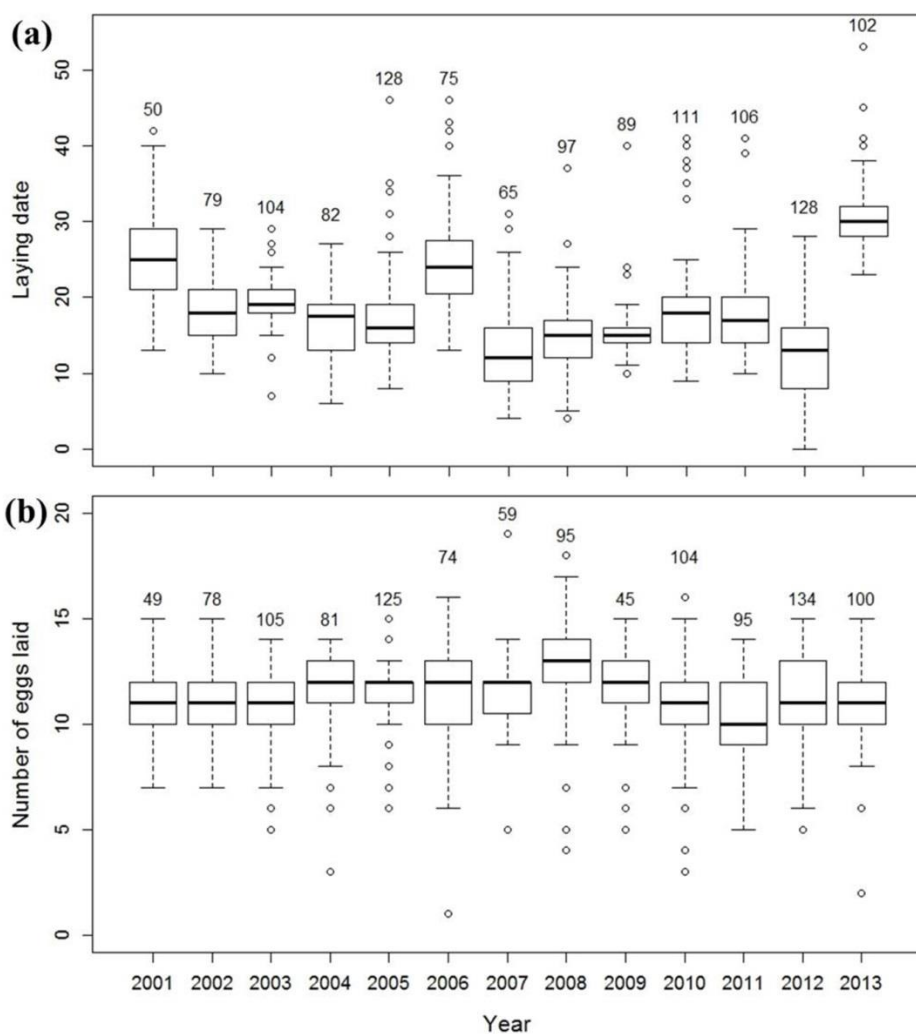


Figure S1 Boxplots of (a) laying date and (b) number of eggs laid in the study area from 2001-2013. Laying dates are given as April dates (1=1 April). Boxes indicate the median, and 25th and 75th percentiles; whiskers indicate the lowest and highest datum within 1.5 times the interquartile range (IQR) from the first and third quartile, respectively. Numbers above the boxes indicate the number of observations.

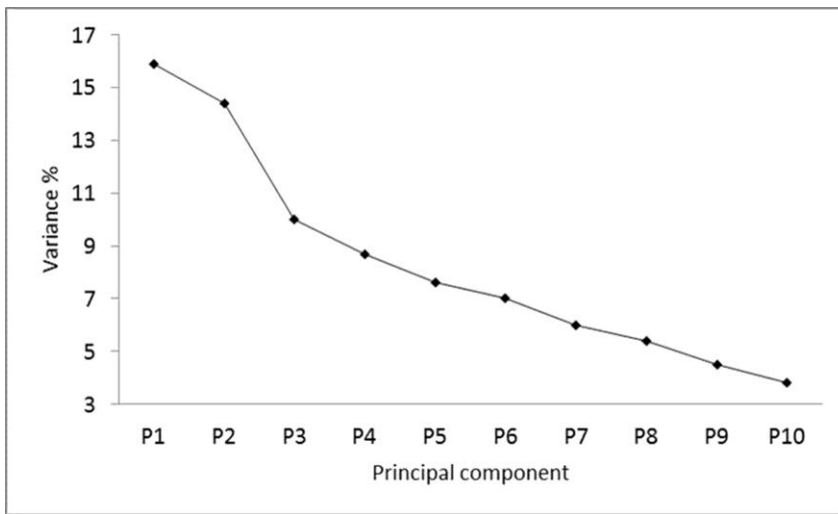


Figure S2 Scree plot for the variance explained by the first ten principal components extracted from the principal component analysis of the vegetation variables.



Photos: Seyed Mehdi Amininasab